Volume 6 Article 23

4-1-1979

Research notes: A partially male-sterile mutant in soybeans

David M. Stelly
Iowa State University

Reid G. Palmer
United States Department of Agriculture

Plant 162-20-17, an M_3 progeny with 39 somatic chromosomes, was a thick-stemmed, vigorous plant that was semi-sterile and matured late. The chromosome number of this plant was confirmed by observations of microsporocytes with 19 bivalents and 1 univalent. Ten progenies of this monosomic plant had 40 chromosomes, the diploid number. More progenies will be grown and their chromosome number determined.

Plant 172-11, an M_2 progeny, appears to carry a reciprocal translocation involving the satellite chromosome. Twenty bivalents were observed in 172-11-3, an M_3 progeny with two short satellite chromosomes and two long chromosomes. Four other M_3 progenies of 172-11 had no observable chromosome aberrations, two had one short satellite and one long chromosomes, and two had one short satellite chromosome. Plant 172-11-3 and the four with no observable chromosome aberrations were fertile whereas the remaining four plants with either a short satellite chromosome or a short satellite chromosome and a long chromosome were late maturing and semi-sterile.

The results indicate that irradiation of soybeans may be as good a method for producing aneuploids as screening asynaptic or desynaptic mutants. A monosomic soybean plant found in the M_3 progeny was of particular interest because hypoploids have not been found among aneuploid progenies from asynaptic or desynaptic mutants.

K. Sadanaga—USDA
R. Grindeland—USDA

IOWA STATE UNIVERSITY
Department of Agronomy
and
UNITED STATES DEPARTMENT OF AGRICULTURE
Ames, IA

1) Spontaneously occurring sterile plants.

Two sterile plants were found in a commercial field of soybeans in Ames in 1977. The plants were noticed because of their retention of chlorophyll when fertile plants had matured and turned brown. One of the plants, 'Sterile A', had set two one-seeded pods, and the other, 'Sterile B', had set 7 seeds.

Other researchers have mentioned or reported the spontaneous occurrence of sterile plants in commercial fields and, commonly, the apparent lack of a genetic determinant for the sterility. Our analysis of descendants of Steriles A and B indicates that genetic sterility is probably lacking in these two cases, also, and that the general occurrence of sterile plants in commercial fields may be due, in part, to ploidic and/or genomic instability.

Progeny of Steriles A and B had elevated chromosome numbers (Table 1) and were highly sterile, except for one plant, D9. D7 was also sterile, except that one branch set several pods. Whether seed formation on Steriles A and B resulted from self- or cross-pollination is not known, since segregation of genetic markers was unexpected.

Chromosome counts of progeny from D7 and D9 revealed low aneuploid chromosome numbers (Table 2), indicating the loss of extra chromosomes and a regression toward the basic 40-chromosome constitution.

Genetic sterility was not evident among the eight viable progeny of D7 or the 28 viable progeny of D9. Reduced seed-set occurred among plants having 42, 43 and 44 chromosomes, as expected, but the lack of sterility among other plants indicated that a recessive or dominant monogenic sterility system had not brought about reduced seed-set on Steriles A and B. Progeny of one 40-chromosome D7 descendant and four 40-chromosome D9 descendants were screened for segregation. Forty-seven to 50 progeny of each plant failed to segregate sterility.

Table 1

Chromosome numbers of progeny from Steriles A and B

Sterile A		Sterile B	
Plant number	Chromosome number	Plant number	Chromosome number
D6	70	D8	68
D7*,†	52	D9†	43
		D10	48
		D11	58
		D12	68
		D13	(Died)
		D14	(Died)

*One axillary branch of D7 was relatively fertile, and set several seeds.
†Progeny of D7 and D9 were analyzed further (see Table 2).

Table 2

Chromosome numbers of D7 and D9 progeny

Chromosome number	D7 progeny	D9 progeny
40	1*	5*
41	1	13
42	3	6
43	2	3
44	1	1
Unknown	2	2

*Progeny of the one D7 descendant and four of the five D9 descendants having 40 chromosomes were screened for segregation of sterility genes in the F_3 generation.

We suspect that the sterility of Steriles A and B resulted from highly elevated chromosome numbers, either euploid or aneuploid. Spontaneous triploids and tetraploids can arise from occasional 2N gametes, and the former are likely to produce highly aneuploid progeny. The data are compatible with this hypothesis, and do not indicate a monogenic system of sterility. We also suspect that the failure of other workers in their attempts to isolate genetic sterility systems readily may be explainable on a similar basis, i.e., cases of sterility in commercial fields could result from abnormal numbers of chromosomes. Plant diseases probably also contribute to the number of naturally occurring sterile plants. The selection of green plants bearing few seed at maturity, therefore, need not lead to the isolation of a genetic sterility system.

David M. Stelly
Patricia S. Muir
Reid G. Palmer—USDA

2) A partially male-sterile mutant in soybeans.

An entry consisting mostly of plants having little to no seed set was found amidst the breeding material of Dr. Walter R. Fehr (Iowa State University) in 1975. The entry was descendent from germplasm population AP6(S1)C1, which was described by Fehr and Ortiz (1975). Investigations have revealed that partial male sterility was the primary cause leading to reduced seed set (Stelly, 1979).

Observations of fresh and paraffin-embedded material manifested the partial male sterility. The ability of partially male-sterile plants to set seed from self-pollination and cross-pollination, and cytological observations, revealed that female fertility is not the factor that limits the amount of seed set by partially male-sterile plants. On the other hand, abnormal female development sometimes occurred, but its incidence was high only among floral buds formed after the regular period of flowering (plants bearing few or no seed continue to flower).

The trait is controlled by a single recessive allele (Table 1). Phenotypic expression of the partial male sterility is highly variable, and subject to modification by background genotype and environment. The amount of selfed seed set on homozygous recessive plants varies considerably, due to incomplete expression of male sterility. When genetically sterile plants set large amounts of seed, they are phenotypically indistinguishable from genetically fertile plants at maturity. Modification of the phenotypes leads to occasional misclassification and, thus, the large homogeneity χ^2 for families shown in Table 1. This interpretation is favored over the alternative explanation that heterogeneity resulted from digenic epistatic inheritance of the trait; progeny tests of fertile F_2 plants gave results expected under the hypothesis of monogenic control, but not digenic control (Table 2).

The gene pleiotropically affects corolla morphology such that standard petals do not bend back, and instead enclose the wing and keel petals. Expression of this floral trait also is variable. Flowering is prolonged when seed set is low; abnormal floral bud differentiation becomes increasingly

Table 1

Segregation of msp msp plants

Segregation[a]	Monogenic inheritance			Homogeneity		
Fertile : Sterile	χ^2	d.f.	P	χ^2	d.f.	P
3289 : 1091	0.0195	1	0.95-0.99	6.12[b]	6	0.25-0.5
				106.58[c]	93	0.075-0.10

[a]Pooled data from segregating F_2 and F_3 families.

[b]Contingency test for homogeneity of populations.

[c]Contingency test for homogeneity of families.

Table 2

Progeny tests of fertile F_2 plants

Type of F_3 family	Chi-squares and probabilities			
Segregating : Nonsegregating	Monogenic[a]	Probability	Digenic[b]	Probability
77 : 39	0.00	1.0-0.9	19.09**	0.00-0.01

[a]If monogenic, the expected ratio of segregating:nonsegregating families from fertile F_2 plants is 2:1.

[b]If digenic with epistasis (i.e., 13:3 F_2 ratio), the expected ratio of segregating:nonsegregating families from fertile F_2 plants is 6:7.

**Significant at the 0.01 probability level.

frequent and fleshy pods are produced as sterile plants age. Plant maturation is normal and vestigial pods are not produced when seed set is normal or nearly normal.

The capacity of the partially male-sterile plants to self-fertilize under certain conditions is reflected by the capacity of homozygous recessive plants to set large numbers of seed and pods and by the preponderance of partially male-sterile plants among the progeny of partially male-sterile seed parents. In some cases, sterile plants have produced more than 100 seeds from self-pollination. The ability of partially male-sterile plants to self-pollinate under certain conditions will allow for the synthesis of large, homogeneous populations of genetically sterile plants, as once suggested by Smith (1947). Such populations will be male sterile if grown in an appropriate environment. The proportion of seed that is cross-pollinated seems to vary

inversely with the total amount of seed set on partially male-sterile plants, but controlled experiments to determine the levels of outcrossing have not been conducted to date. A large population of partially male-sterile plants homozygous for w_1 is being generated, however, for this purpose.

This mutant line has been assigned Genetic Type Collection T-number T271H and the gene symbol msp by the Soybean Genetics Committee.

References

Fehr, W. R. and L. B. Ortiz. 1975. Registration of a soybean germplasm population. Crop Sci. 15: 739.

Smith, L. 1947. Possible practical method for producing hybrid seed of self-pollinated crops through the use of male sterility. J. Amer. Soc. Agron. 39: 260-261.

Stelly, D. M. 1979. Investigations of a partially male-sterile line of soybeans, Glycine max (L.) Merrill. M.S. Thesis. Iowa State University, Ames, Iowa, USA.

David M. Stelly
Reid G. Palmer—USDA

3) A cytologically identifiable short chromosome.

Seeds set on partially male-sterile plants (see article 2 for a description of the sterility system) were grown in the greenhouse in the spring of 1977. One of the plants, designated D56, had an unusual growth habit--the plant was somewhat spindly, climbing, and had a thin main stem. It had been noted at the time of transplanting that the root system of the D56 seedling consisted of a very long tap root and unusually thin lateral roots. Petiole cuttings were made in order to check the chromosome number of D56, but we were unable to establish the chromosome number of the plant. Subsequent pollen sampling revealed semi-sterility among pollen grains (41.7% of the pollen grains were aborted, i.e., they did not stain in I_2KI). Ovule abortions were frequent also, giving further evidence of gametophyte inviability.

The exact source of the semi-sterility is unknown, since D56 resulted from a natural cross-pollination. But several of our observations indicate that G. soja, "G. gracilis", or an introgression product of one of these species with G. max, was the male parent of D56. First, the growth habit of D56 was more like that of "G. gracilis" than of G. max. Second, D56 was heterozygous for L_1 (black pod) and homozygous for T (tawny pubescence); the partially male-sterile female parent was l_1l_1TT, and the only L_1L_1TT material grown in the field in 1976 was descendent from Plant Introductions of G. soja and "G. gracilis". Third, seeds formed by D56 were somewhat small, their seed coats were an off-yellow color (perhaps an indirect effect of L_1) and their dark hila were uniformly ringed by a narrow region of the seed coat that was pigmented; this sort of seed pigmentation normally is not observed in G. max x G. max crosses. Fourth, segregation of F_2 genotypes led to an array of seed coat colors on F_3 seed (maternal tissue), including the dark, speckled seed coat found in G. soja and "G. gracilis". Thus, we are reasonably confident

that the semi-sterility, and the short chromosome described below, came from one of these species or from an introgression product.

Root tips from seeds produced by D56, later generations and testcrosses have been used to determine chromosome numbers. Analysis of D56 progeny revealed numerous cases of aneuploidy. Just as important, the presence of an abnormally short chromosome was noted. The small chromosome is roughly one-half of the size of the smallest chromosome in the Glycine max complement; it is slightly sub-metacentric. The chromosome is readily identifiable in well-spread mitotic metaphases.

In addition to identifying a variety of aneuploid conditions (Table 1) that involve only the short chromosome, we have found several aneuploid plants whose aneuploidy involved one or more univalent shifts. Rate of transmission of the small chromosome has been high among self-progeny, and moderately so in cross-pollinations. Our data concerning transmission of the larger trisomic chromosomes (from univalent shifts) are presently too limited to allow an inference on the rate(s) of transmission for that/those chromosome(s).

Table 1

Types of chromosome constitutions that occurred among the progeny of D56

Chromosome number	Type of extra chromosome			
	None	One short	Two short	One normal*
40	+	+	-[a]	-
41		+	-[b]	+
42		+	+	+

*'Normal', referring to a chromosome that was not short.

[a]We have screened a few progeny from plants having 39 normal and one short chromosomes, but have not recovered plants with 38 normal and two short chromosomes.

[b]We have found plants with 39 normal and two short chromosomes among progeny from plants having 39 normal and one short chromosome.

Preliminary analysis of meiosis has indicated that the small chromosome is often present as a univalent at metaphase 1 in PMC's, and as cytoplasmic bodies in tetrads. Quantitative data have not been collected yet. We have not observed configurations suggesting the presence of a translocation, deletion, or inversion, to date, though either of the first two types of aberrations might have been involved in the formation of the small chromosome. Certain features of the distributions of pollen and ovule abortions across karyotypes suggest that such an aberration may be segregating in the material.

Our work presently involves determining rates of transmission for the small chromosome and its derivatives resultant from univalent shifts, testing for homology among the new aneuploids, and between the new aneuploid(s) and

Trisomics A, B and C. Studies of the meiotic behavior of the chromosomes will be included.

David M. Stelly
Hollys E. Heer
Reid G. Palmer—USDA

4) Seed coats of Glycine soja and "G. gracilis"--inheritance of color/pattern.

In the preceding note, it was mentioned that the derivation of an abnormally short chromosome involved natural cross-pollination of a partially male-sterile (msp msp) plant (A76-517-2) by pollen from G. soja, "G. gracilis", or an introgression product of these species into G. max. The recessive allele for self seed coat color (i) and the allele(s) producing the dark seed coat pattern of G. soja and "G. gracilis" were concomitantly transferred in the cross-pollination. Segregation in later generations and a few testcrosses indicate that the characteristically patterned seed coats of G. soja and "G. gracilis" are governed by an allele of the R locus; the allele appears to be dominant to r (brown), r^m (ring-pattern) and, perhaps, to R (black). For the purpose of this note, however, we will refer to the patterned seed coat as the 'soja-type'.

We have observed segregation of the soja-type seed coat in families descendent from parents having the soja-type or yellow seed coats, but not in families descendent from plants having brown seed coats. This led us to believe that the patterned seed coats of G. soja and "G. gracilis" might be dependent on the presence of an r allele. Limited data from F_2 plant segregation are compatible with this hypothesis (Table 1).

Table 1

Segregation of plants having either soja-type or brown seed coats

Generation	Segregation soja-type : brown	d.f.	χ^2	Probability
F_2	23 : 8	1	0.0107	
F_2	21 : 6	1	0.1111	
Sum	44 : 14	1	0.0229	0.9-0.95

One hybrid plant was produced from a cross-pollination of an $ii\,r^m r^m$ (T125) plant with pollen from a plant having the soja-type seed coat. The hybrid produced F_2 seed having the soja-type seed coat (maternal tissue), indicating that the soja-type allele is dominant over r^m. F_2 plants will be grown in the summer of 1979, and their seed classified for seed coat color/pattern.

Expression of the soja-type seed coat is dependent on the lack of I. All F_1 plants from crosses between plants having the soja-type seed coat color and those homozygous for I produced seeds with yellow or green seed coats. In two crosses between the soja-type and plants of the $I\,I\,T\,T\,W_1W_1\,R\,R$ genotype (yellow seed coat and gray hilum), F_1 plants produced seeds with yellow seed coats and dark hila.

Further information on the allelism and order of dominance for the soja-type seed coat will be obtained as additional testcross and segregation data are collected.

David M. Stelly
Reid G. Palmer—USDA

5) A new chlorophyll mutant.

A new recessive chlorophyll mutant was unexpectedly recovered in an F_2 family of A76-518-3 (a homozygous partially male-sterile, msp msp, plant) x A76-669 (a 'Clark' isoline homozygous for the chromosome translocation from PI 101,404B). Furthermore, the translocation did not appear in the F_2 generation. We are uncertain as to whether the intended cross was unsuccessful and followed by a natural outcross, or if a new chromosomal rearrangement had taken place. The former seems more likely. The F_2 population segregated the partial male sterility trait, so we are certain that a cross was involved.

Field-grown plants homozygous for the mutant allele first manifest abnormal chlorophyll content as seedlings; progressive chlorosis and necrosis sometimes kills seedlings, but others survive as short spindly plants. This mutant differs from T265H in that yellow plants often survive both in the field and greenhouse. Shading from healthy (green) sibs seems to promote the health of the chlorotic seedlings and plants. Seedlings which survive and flower often set a few seed. We have noted that the amount of shading given to plants in the greenhouse also affects the longevity of the mutants. Temperature, too, may influence viability of the plants; one mutant plant that was grown in a shaded region of a cool greenhouse remained relatively healthy and produced a large number of seed.

A further indication that environment affects expression of the allele comes from the observation of seedlings grown in a greenhouse sandbench. Initial screening of F_3 families was done in the greenhouse during the winter of 1977-1978. Five seeds from each of 48 F_3 families were sown and grown to the three-trifoliolate leaf stage. Chlorosis was not observed among any of the families grown in the sandbench, but was observed among field-grown sibs. Low light intensity in the greenhouse during the winter months may have precluded expression of the chlorosis.

Data from F_2 segregation of the new mutant are compatible with the hypothesis of monogenic recessive control of the mutant phenotype (Table 1). F_3 data, however, are only marginally compatible with the same hypothesis, due to a relative deficiency of mutant phenotypes (Table 1). Although F_3 families were homogeneous for their ratios of segregation, the overall deficiency of mutant phenotypes warranted tests for the possibility of digenic epistatic inheritance.

Table 1

Segregation data for a chlorophyll-deficient phenotype

Generation (year)		Segregation Normal : Yellow	Monogenic recessive χ^2	Monogenic recessive Probability	Homogeneity d.f.	Homogeneity χ^2	Homogeneity Probability
F_2	1977	92 : 34	0.265	0.75-0.50	-	-	-
F_3[a]	1978	1118 : 327	4.322*	0.05-0.025	36	39.75	0.5-0.25

[a]Data from segregating families only.

*Significant at the 0.05 probability level.

F_3 families from F_2 green plants included 37 segregating families and 11 nonsegregating (all green) families. These results are compatible with the hypothesis of monogenic recessive inheritance for the yellow phenotype, but are incompatible with the hypothesis of digenic epistatic control (Table 2). We conclude, therefore, that a single mutant recessive allele controls the chlorotic phenotype. The mutant has been assigned soybean genetic type collection T-number (T270H), but has not been assigned a gene symbol, due to the possibility of allelism with previously designated alleles that are maintained at Urbana, Illinois.

Table 2

F_3 analysis of green F_2 plants

Generation	Segregating : Nonsegregating	Chi-squares and probabilities Monogenic[a]	P	Digenic[b]	P
F_3 families	37 : 11	2.344	0.25-0.10	18.487**	0.01-0.00

[a]If monogenic, the expected ratio of segregating : nonsegregating families from green F_2 plants is 2 : 1.

[b]If digenic with epistasis (i.e., 13 : 3 F_2 ratio), the expected ratio of segregating : nonsegregating families from green F_2 plants is 6 : 7.

**Significant at the 0.01 probability level.

David M. Stelly
Patricia S. Muir
Reid G. Palmer—USDA

6) Inheritance and expression of a mutant phenotype affecting the number of petals per flower.

Plants of the Glycine max Plant Introduction 68,704 characteristically produce flowers that have six or more petals, rather than the normal complement of five petals (1 standard, 2 wing, and 2 keel petals). We have investigated the inheritance and expression of this trait.

Eight F_1 plants were classified by sampling ten flowers per plant; none of the F_1 plants produced more than five petals, indicating that the phenotype is under recessive genetic control. Since all of the crosses employed PI 68,704 as female and L15 as male, we cannot eliminate the possibility of a cytoplasmic interaction with nuclear control.

Data from F_2 segregation indicate that the trait is controlled digenically and that plants homozygous for recessive alleles at either locus can produce flowers having more than five petals (Table 1).

The production of extra petals by mutant plants is a variably expressed trait; normal plants produce extra petals only very rarely. In plants of this Plant Introduction, every flower seems to be affected, albeit variably. A sampling of 10 flowers from each of 9 plants yielded no instance where only five petals were present. Extra wing and keel petals occurred more frequently than did extra standard petals (Table 2). In contrast, the level of expression was much more erratic among flowers of mutant plants that segregated in the F_2 families; many flowers contained only the normal complement of petals. The distribution of extra petals among the different petal types seems to have been altered; also the number of extra wing and standard petals were similarly low, but the number of extra keel petals remained relatively high (Table 2).

Whether or not incomplete epistasis accounts for all or part of the differences observed between plants of this Plant Introduction and F_2 families can be tested through statistical analysis of expression on mutant plants having known genotypes. Such plants will become available as backcrosses and testcrosses are made.

Table 1

Data from F_2 plant segregation of normal and mutant plants, from the cross PI 68,704 x L15

	Segregation		
	Normal : Mutant	$\chi^2$1 d.f.	Probability
Observed	83 : 56		
Exp. (3:1)[a]	104.25 : 34.75	17.326	0.0-0.005
Exp. (9:7)[b]	78.19 : 60.81	0.677	0.25-0.50

[a]Segregation ratio expected under monogenic recessive control.

[b]Segregation ratio expected under digenic recessive control, where the recessive condition at either locus is epistatic to dominant alleles at the other locus.

Table 2

Mean number of extra petals per flower, by types of petals, for parental, F_1 and F_2 plants

Line	Petal types			Average
	Keel	Wing	Standard	
PI 68,704	0.933 (0.067)[a]	0.755 (0.073)	0.300 (0.053)	0.663 (0.041)
L15	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F_1	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F_2 normals	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
F_2 mutants	0.350 (0.0296)	0.082 (0.015)	0.041 (0.084)	0.158 (0.012)

[a]Standard errors of means are given parenthetically.

David M. Stelly
Reid G. Palmer—USDA

7) Reference diagrams of seed coat colors and patterns for use as genetic markers in crosses.

Specht and Williams (1978) reported on the use of hilum color as a genetic marker in soybean crosses. We present the classification of seed coat color and seed coat patterns that we have been using. Table 1 lists the genes affecting seed coat pigmentation to be considered. Table 2 presents data from the 64 genotypic combinations according to flower and pubescence color. Table 3 summarizes the data.

Table 1

Genes affecting seed coat pigmentation

Gene	Phenotype	Gene	Phenotype
I	light hilum	R	black seed
i^i	dark hilum	r	brown seed
i^k	saddle	T	tawny (brown) pubescence
i	self dark color	t	gray pubescence
O	brown seed	W_1	purple flower
o	reddish brown seed	w_1	white flower

Table 2

Genotypic combinations for seed coat, saddle and hilum colors

	TW and Tw*				tW					tw			
	RO	Ro	rO	ro		RO	Ro	rO	ro		RO	Ro	rO	ro
I	G**	G	Y	Y	I	G	G	Y	Y	I	Y	Y	Y	Y
i^i	Bl	Bl	Br	Rbr	i^i	Ib	Ib	Bf	Bf	i^i	Bf	Bf	Bf	Bf
i^k	Bl	Bl	Br	Rbr	i^k	Ib	Ib	Bf	Bf	i^k	Bf	Bf	Bf	Bf
i	Bl	Bl	Br	Rbr	i	Ib	Ib	Bf	Bf	i	Bf	Bf	Bf	Bf

*See Table 1 for complete description of T, t, W, w, R, r, O, o, I, i^i, i^k and i.

**G = gray, Bl = black, Br = brown, Rbr = reddish brown, Y = yellow, Ib = imperfect black, Bf = buff. Seed coat color is yellow or nearly so in I and i^i genotypes and matches the hilum color in i genotypes. Saddle color (i^k genotypes) also matches the hilum color.

Table 3

Summary of 64 genotypic combinations for seed coat, saddle and hilum colors

Genes				Phenotypes			
				Self color	Saddle & hilum color	Hilum color	Hilum color
				i	i^k	i^i	I
T	R			black	black	black	gray
T	r	O		brown	brown	brown	yellow
T	r	o		reddish brown	reddish brown	reddish brown	yellow
t	R		W_1	imperfect black	imperfect black	imperfect black	gray
t	R		w_1	buff	buff	buff	yellow
t	r			buff	buff	buff	yellow

The use of genetic markers for distinguishing between hybrid and 'self' progeny is even more important when making cross-pollinations (Walker et al., 1979). As Specht and Williams (1978) have pointed out, hilum and seed coat colors may be used as genetic markers when flower, pubescence and pod color are not useful markers.

Seed coat and hilum phenotypes corresponding to combinations of alleles at five gene loci (I, R, O, T and W) are presented in this report. The O locus was not considered by Specht and Williams, but it, too, can be employed

as a genetic marker for checking cross-pollination success. The O and I gene loci are linked, with 17.8 + 0.7% recombination (Weiss, 1970); segregation at O and I loci may generate unexpected phenotypes in certain crosses.

References

Specht, J. E. and J. H. Williams. 1978. Hilum color as a genetic marker in soybean crosses. Soybean Genet. Newsl. 5: 70-73.

Walker, A. K., S. R. Cianzio, J. A. Bravo and W. R. Fehr. 1979. Comparison of emasculation and non-emasculation for hybridization of soybeans. Crop Sci. In press.

Weiss, M. G. 1970. Genetic linkage in soybeans: Linkage Group VII. Crop Sci. 10: 627-629.

R. G. Palmer—USDA
D. M. Stelly

8) A flower structure mutant.

A flower structure mutant was found segregating within the original heterogeneous PI 339,868 population in 1970. This mutant is characterized by having cleistogamous flowers with an exposed stigma and is sterile.

The flowers of sterile plants have been observed by dissections, serial paraffin sections and the scanning electron microscope (SEM). Flower development and structure were found to be abnormal. The petals of these flowers grow abnormally and eventually surround the stamens. Consequently, staminal tube elongation is blocked. At anthesis the anthers are positioned around the ovary rather than around the stigma, and the petals are curved over the top of the anthers. Self-pollination is prevented by the spatial separation between the anthers and stigma and by the physical barriers of the petals.

The actual cause of sterility in this mutant has not been determined. It is not male sterile. Pollen grains produced by sterile plants stain normally with I_2KI and frequently have been observed germinating *in vivo* with the SEM and in paraffin serial sections.

This mutant may have some degree of female sterility. Megasporogenesis, observed in paraffin serial sections, looks normal. However, in 200 hand pollinations attempted, using the sterile plant as female, only 1 seed was produced. This lack of crossing success could be due to the absence of normal indicators of female receptiveness (petal size and color) or it could be due to the exposed stigma drying out before the female is receptive. On the other hand, the lack of crossing success using the mutant as female could be a result of female sterility.

The fact that self pollination does not take place has been documented by dissections, serial paraffin sections and SEM. This, by itself, could lead to sterility in this mutant. However, further study is needed to determine the degree of female sterility and the contribution it makes to sterility in this mutant.

Segregation for fertility : sterility within PI 339,868 is 3 : 1 (Table 1). However, when this parent population is crossed to genetically unrelated populations, the resulting F_2 ratio in segregating families is 15 fertile plants to every sterile plant (Table 2). These ratios indicate that the sterility is controlled by two genes and that both genes must be homozygous recessive to produce a sterile plant. From the data in Tables 1 and 2, we conclude that PI 339,868 is homozygous recessive for one gene and segregating for the second gene.

This sterile was tested for linkage with several other traits. To date, no linkage has been detected (Table 3). Other linkage tests are in progress.

This mutant has been designated $\underline{fs_1}\,\underline{fs_1}\,\underline{fs_2}\,\underline{fs_2}$ (flower structure) and has been given Genetic Type Collection Number T269 by the Soybean Genetics Committee. Thus, the original PI 339,868 population is considered to be $\underline{Fs_1}\,\underline{fs_1}\,\underline{fs_2}\,\underline{fs_2}$ and will be maintained as T269H.

Table 1

Segregation within PI 339,868

	Family					Plant				
Year	Total	Seg.	Not seg.	χ^2(2:1)	P	Total	Fertile	Sterile	χ^2(3:1)	P
1971	12	8	4	0.000	1.00	176	136	40	0.485	<.50
1972	84	60	24	0.857	<.50	2048	1532	516	0.417	<.75
1974	86	57	29	0.006	<.96	2168	1637	531	0.298	<.75

Table 2

F_2 segregation in crosses with PI 339,868

Populations crossed to	Total plants	Segregation		χ^2(15:1)	P
		Fertile	Sterile		
Hark	1189	1105	84	1.348	<.25
Clark	437	408	29	0.112	<.75
Clark T/T	717	670	47	0.114	<.75
T93	813	752	61	2.176	<.25
T219H	361	336	25	0.282	<.75
T230	211	198	13	0.003	<.975
T241	2555	2396	159	0.003	<.975
T242	1974	1845	129	0.273	<.75
T258	1201	1114	87	2.026	<.25

Table 3

Linkage tests with PI 339,868

Trait tested	Segregation				Expected ratio	χ^2	P
	Fertile		Sterile				
Flower color	W	w	W	w			
	1023	332	76	30	45:15:3:1	3.41	<.50
Pubescence color	T	t	T	t			
	827	283	60	26	45:15:3:1	3.39	<.50
Clark translocation							
Normal/50% aborted	Normal	50%	Normal	50%			
	349	321	19	28	15:15:1:1	3.087	<.50
Trisomic C							
40 chromosomes	480		36		15:1	0.465	<.50
41 chromosomes	625		48		15:1	0.895	<.50
T241 ($\underline{st_2}$)†	2424		652		51:13	1.485	<.25
T242 ($\underline{st_3}$)†	1244		316		51:13	0.003	<.975
T258 ($\underline{st_4}$)†	2177		530		51:13	0.900	<.50

†In F_2 populations segregating both loci from PI 339,868 and $\underline{st_2}$, $\underline{st_3}$ or $\underline{st_4}$, we expect a 153:19:16:4 ratio. Since all sterile genotypes have identical phenotypes at maturity, all sterile plants are grouped, producing a 153:39 ratio (simplified to 51:13).

Carol L. Winger
Reid G. Palmer—USDA

9) Genetics of the meiotic mutant $\underline{st_5}$.

In 1970, a part-sterile plant in Uniform Test I, entry W6-4108 (from Wisconsin), was observed at Ames, Iowa. Seven seeds from this part-sterile plant gave rise to seven plants in 1971; six were fertile and one was sterile and set no seeds. In 1972, five plant progeny rows gave all fertile plants, i.e., they did not segregate fertile and sterile plants. Plant progeny row A72-441-3 segregated 30 fertile : 13 sterile plants. Twenty-two plant progeny rows were planted in 1973; 14 segregated both fertile and sterile plants and 8 had only fertile plants. Table 1 summarizes the frequencies of fertile and sterile plants in segregating F_2 families. The 122 sterile plants set no seed.

Pollen grains from the Wisconsin sterile were stained with I_2KI. Pollen grains were small, shrunken and collapsed, and were similar in appearance to pollen grains from $\underline{st_2}$, $\underline{st_3}$ and $\underline{st_4}$ plants. Microspore mother cells of sterile plants were examined, and a low level of chromosome pairing was observed, indicating that the sterile was either an asynaptic or desynaptic mutant.

Three nonallelic asynaptic or desynaptic mutants have been reported previously in soybeans. Hadley and Starnes (1964) reported $\underline{st_2}$ (T241) and $\underline{st_3}$ (T242) and Palmer (1974) described $\underline{st_4}$ (T258). Winger *et al.* (1977) described a spontaneous mutant at the $\underline{st_2}$ locus.

The purpose of this study was to determine if this new asynaptic or desynaptic mutant, the Wisconsin sterile, $\underline{st_?}$, is allelic to either $\underline{st_2}$, $\underline{st_3}$ or $\underline{st_4}$. This was accomplished by crossing known heterozygotes, i.e., $\underline{St_2st_2}$ x $\underline{St_?st_?}$, $\underline{St_3st_3}$ x $\underline{St_?st_?}$ and $\underline{St_4st_4}$ x $\underline{St_?st_?}$. F_1 and F_2 populations of each cross were observed. If two lines were allelic with regard to their sterility, then one out of four F_1 plants would be sterile; in the F_2 generation, nonsegregating families and families segregating 3 fertile : 1 sterile plants would be observed. If different genes were controlling sterility in the two lines, however, no sterile plants would be observed in the F_1 generation. Moreover, the F_2 generation would include nonsegregating families, families segregating 3 fertile : 1 sterile plants, and families segregating 9 fertile : 7 sterile plants.

No sterile plants were found among F_1 plants from the three genetic combinations of T241H, T242H and T258H with the Wisconsin sterile, respectively. Among segregating F_2 populations, two groups were evident on the basis of the Chi-square values (Tables 1, 2, 3 and 4). One group seemed to represent a 3:1 population; the other group seemed to represent a 9:7 population. These results agree with the hypothesis that the recessive gene in the Wisconsin sterile is different from the genes in T241, T242 or T258. As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T272) and the gene symbol $\underline{st_5}$ by the Soybean Genetics Committee. This line is maintained as the heterozygote, T272H.

References

Hadley, H. H. and W. J. Starnes. 1964. Sterility in soybeans caused by asynapsis. Crop Sci. 4: 421-424.

Palmer, R. G. 1974. A desynaptic mutant in the soybean. J. Hered. 65: 280-286.

Winger, C. L., R. G. Palmer and D. E. Green. 1977. A spontaneous mutant at the $\underline{st_2}$ locus. Soybean Genet. Newsl. 4: 36-42.

Table 1

Frequencies of fertile and sterile plants in segregating F_2 families of the Wisconsin sterile (W6-4108)

Year	Fertile	Sterile	χ^2(3:1)	P
1973	377	122	0.08	<0.90

Table 2

Ratio of fertile to sterile plants in segregating[a] F_2 families from crosses between heterozygous plants of T258 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	χ^2 (3:1)	P	Fertile plants	Sterile plants	χ^2 (9:7)	P
Totals	588	196	5.24	<0.90	80	59	0.18	<0.75
Pooled χ^2			0.00	0			0.10	<0.90
Homogeneity χ^2			5.24	<0.90			0.08	<0.90

[a]10 families appeared to segregate 3:1 and 2 families 9:7.

Table 3

Ratio of fertile to sterile plants in segregating[a] F_2 families from crosses between heterozygous plants of T242 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	χ^2(3:1)	P	Fertile plants	Sterile plants	χ^2(9:7)	P
Totals	1138	385	9.56	<0.99	459	336	3.05	<0.975
Pooled χ^2			0.06	<0.90			0.71	<0.50
Homogeneity χ^2			9.50	<0.975			2.34	<0.975

[a]20 families appeared to segregate 3:1 and 9 families 9:7.

Table 4

Ratio of fertile to sterile plants in segregating[a] F_2 families from crosses between heterozygous plants of T241 and heterozygous plants of the Wisconsin sterile

	Fertile plants	Sterile plants	χ^2 (3:1)	P	Fertile plants	Sterile plants	χ^2 (9:7)	P
Totals	1169	362	15.02	<0.90	215	147	3.52	<0.75
Pooled χ^2			1.50	<0.25			1.45	<0.25
Homogeneity χ^2			13.52	<0.95			2.07	<0.75

[a]23 families appeared to segregate 3:1 and 5 families 9:7.

Reid G. Palmer—USDA

10) Inheritance of male-sterile, female-fertile mutant $\underline{ms_3}$.

Two non-allelic male-sterile strains each controlled by a single recessive gene, $\underline{ms_1}$ (Brim and Young, 1971) and $\underline{ms_2}$ (Bernard and Cremeens, 1975), respectively, have been reported in soybeans. We now have evidence for a third completely male-sterile type controlled by a single recessive gene at a different locus from either $\underline{ms_1}$ or $\underline{ms_2}$. As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T273) and the gene symbol $\underline{ms_3}$ by the Soybean Genetics Committee. This line is maintained as the heterozygote T273H.

In 1971, in an F_3-derived line from the cross 'Calland' x 'Cutler', Dr. John Thorne of Northrup, King & Co., Washington, Iowa, observed several sparsely podded plants. Fertile plants in this plant progeny row were harvested and evaluated in 1972. In segregating families, we found approximately 3 fertile : 1 sterile plants (529:183, expected 534:178).

Sterile plants had normal-appearing anthers but pollen grains were poorly stained with I_2KI and were slightly smaller than pollen grains from fertile plants. Microspore mother cells of sterile plants were examined; meiosis was normal. As soon as the microspores were released from the tetrad, however, they began to abort. Pollinations were made on sterile plants with a success rate nearly as high as on fertile plants (51% pod set versus 56% pod set, respectively).

In order to test the relationship of the Northrup, King male-sterile to $\underline{ms_1}$ and $\underline{ms_2}$, we made crosses using male-steriles as the female parent and heterozygotes as the male parent (Tables 1 and 2). All F_1 plants were fertile. In the F_2, as would be expected if $\underline{ms_1}$ or $\underline{ms_2}$ and the Northrup, King male sterile were at separate and unlinked loci, half of the families segregated 3:1 and half segregated 9:7 (Tables 1 and 2).

The inability to identify male-sterile plants before flowering severely restricts use of this mutant in commercial hybrid seed production, but this mutant may be useful in genetic or plant breeding experiments.

Table 1

Male-sterile allelism tests between $\underline{ms_1ms_1}$ and Northrup, King male sterile

	$\underline{ms_1ms_1}$ x T273H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	χ^2	Total fertile	Total sterile	d.f.	χ^2
Totals	1033	351	7	2.02	899	699	6	1.51
Pooled χ^2 (1 d.f.)			1	0.10			1	0.00
Homogeneity χ^2			6	1.92			5	1.51

Table 2

Male-sterile allelism tests between $\underline{ms_2ms_2}$ and Northrup, King male sterile

	$\underline{ms_2ms_2}$ x T273H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	χ^2	Total fertile	Total sterile	d.f.	χ^2
Totals	505	168	10	2.29	315	251	6	6.68
Pooled χ^2 (1 d.f.)			1	0.01			1	0.08
Homogeneity χ^2			9	2.28			5	6.60

References

Bernard, R. L. and C. R. Cremeens. 1975. Inheritance of the Eldorado male-sterile trait. Soybean Genet. Newsl. 2: 37-39.

Brim, C. A. and M. F. Young. 1971. Inheritance of a male-sterile character in soybeans. Crop Sci. 11: 564-566.

Reid G. Palmer—USDA

11) Inheritance of male-sterile, female-fertile mutant $\underline{ms_4}$.

The previous article mentioned male-sterile mutants $\underline{ms_1}$ and $\underline{ms_2}$ and described the genetics of a new male-sterile mutant, $\underline{ms_3}$. We now have evidence for a fourth completely male-sterile type controlled by a single recessive gene at a different locus from either $\underline{ms_1}$, $\underline{ms_2}$ or $\underline{ms_3}$. As a result of the present study, this mutant was assigned a Genetic Type Collection T-number (T274) and the gene symbol $\underline{ms_4}$ by the Soybean Genetics Committee. This line is maintained as the heterozygote T274H.

In 1973, one sparsely podded plant was observed in a field of 'Rampage' grown at the Bruner Farm near Ames, Iowa. All three progeny from this plant were grown in the greenhouse and were fertile. Plant progeny rows were grown in 1974. One plant row was completely fertile, one plant row segregated both fertiles and steriles and tawny and grey pubescence; one plant row segregated fertiles and steriles and was similar in appearance to Rampage. This last plant row, A74-4646-2, is the source of the Rampage male sterile.

Fertile plants in plant progeny row A74-4646-2 were harvested and evaluated in 1975. In segregating families we found approximately 3 fertile : 1 sterile plants (640:208, expected 636:212). Sterile plants had normal-appearing anthers but pollen grains clumped and stained poorly with I_2KI. Microspore mother cells of sterile plants were not examined. Pollinations were made on sterile plants with a success rate as high as on fertile plants (52% pod set versus 49% pod set, respectively).

In order to test the relationship of the Rampage male sterile to $\underline{ms_1}$, $\underline{ms_2}$ and $\underline{ms_3}$, we made crosses using male steriles as the female parent and heterozygotes as the male parent (Tables 1, 2 and 3). All F_1 plants were fertile. In the F_2, as would be expected if $\underline{ms_1}$ or $\underline{ms_2}$ or $\underline{ms_3}$ and the Rampage male sterile were at separate and unlinked loci, half of the families segregated 3:1 and half segregated 9:7 (Tables 1, 2 and 3).

As is the situation with $\underline{ms_1}$, $\underline{ms_2}$ and $\underline{ms_3}$, the inability to identify male-sterile plants before flowering severely restricts use of these mutants in commercial hybrid seed production.

Table 1

Male-sterile allelism tests between Rampage male sterile and $\underline{Ms_1ms_1}$

	T274 x $\underline{Ms_1ms_1}$							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	χ^2	Total fertile	Total sterile	d.f.	χ^2
Totals	755	242	7	3.56	453	327	6	3.55
Pooled χ^2 (1 d.f.)			1	0.91			1	1.06
Homogeneity χ^2			8	2.65			7	2.49

Table 2

Male-sterile allelism tests between $\underline{ms_2ms_2}$ and Rampage male sterile

	$\underline{ms_2ms_2}$ x T274H							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	χ^2	Total fertile	Total sterile	d.f.	χ^2
Totals	930	303	8	2.42	873	723	6	8.79
Pooled χ^2			1	0.12			1	1.56
Homogeneity χ^2			9	2.30			7	7.23

Table 3

Male-sterile allelism tests between Rampage male sterile and $\underline{Ms_3ms_3}$

	T274 x $\underline{Ms_3ms_3}$							
	3:1 segregation				9:7 segregation			
	Total fertile	Total sterile	d.f.	χ^2	Total fertile	Total sterile	d.f.	χ^2
Totals	1326	421	13	8.40	984	786	8	3.43
Pooled χ^2			1	0.76			1	0.31
Homogeneity χ^2			14	7.64			9	3.12

Reid G. Palmer—USDA

KOREA ATOMIC ENERGY RESEARCH INSTITUTE
P.O. Box 7, Cheongryang-Ri, Seoul, Korea

1) Inheritance of resistance to necrotic strain of SMV in soybean.

A necrotic strain of soybean mosaic virus (SMV) is one of the most destructive diseases in some leading soybean cultivars of Korea and its infection sometimes causes complete loss of the crop. The necrotic disease reported first as a strain of soybean mosaic virus in 1976, affects the most promising commercial cultivars, 'Kwangkyo' and 'Gangrim', which have been cultivated extensively since released in 1969. Hence, an investigation on the mode of inheritance of resistance gene in soybean cultivars was undertaken to develop resistant lines to the necrotic virus disease by mutation technique, which is being carried out with the cultivar Kwangkyo at present.

Paschal and Goodman (1978) reported resistance to a severe isolate of soybean mosaic virus in cultivar 'Buffalo' to be conditioned by one or more dominant genes. Three resistant soybean cultivars and a Korean native line were engaged to cross with the susceptible cultivar Kwangkyo. The F_1 plants for each of the four crosses were grown in the field, and flower, pubescence and seed coat colors were used as genetic markers to verify the hybridization. Both F_1, F_2 plants and parents were grown in the field and inoculated with extract of infected leaves by conventional rubbing method at 2-4 leaf stage, being put aphids to enhance natural infection, too.

The F_1 hybrids of each cross between Kwangkyo and #31926, KEX-2, 'Kumgang-daerip', KAS 390-10 were susceptible, indicating that resistance is controlled by recessive gene (Table 1). In determination of disease reactions of the F_2 populations, it was segregated in a ratio of 3 susceptible to 1 resistant, thus confirming that resistance is conditioned by a single recessive gene. For further evidence, backcrosses and F_3 generations are to be